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Human gastric intrinsic factor expression is not restricted to parietal cells

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ABSTRACT

Gastric parietal cells have been accepted as the only site of intrinsic factor production in the human stomach. In animals, however, intrinsic factor has been localised to various other cell types of foregut origin, including chief and enteroendocrine cells in gastric mucosa, and duct cells from salivary glands and pancreas. The availability of recombinant human intrinsic factor has led to production of high titre, monospecific antiserum which was used to reexamine the distribution and subcellular localisation of intrinsic factor in the human stomach. Immunolight microscopy revealed that most positively stained cells were gastric parietal cells, but at the margins of the anatomical regions (e.g. cardia/fundus, body/antrum) clusters of gastric chief cells and individual enteroendocrine cells were found to contain intrinsic factor. Immunoelectron microscopy demonstrated the highest antigen density on endocytic and apical membranes of parietal cells. Exocrine secretory granules of a subpopulation of chief cells, the secretory granules of some enteroendocrine cells, and the plasma membranes and smooth vesicles of endothelial cells of the lamina propria capillaries underlying enteroendocrine cells were also positive for the antigen. Labelling in all cells was specific, as it was abolished by preabsorption of the antisera with purified recombinant human intrinsic factor. These findings demonstrate a potential for cellular expression of human intrinsic factor in nonparietal cells. Because such expression occurs normally at the margins of anatomical gastric regions, it suggests that local factors may influence expression of intrinsic factor.

Key words: Stomach; chief cells; enteroendocrine cells.

INTRODUCTION

A number of different cell types have been demonstrated by a variety of methods to be the site of production of gastric intrinsic factor (IF) in mammals. Using a radioautographic method binding radioactive cobalamin and blocking the binding with IF antibodies, IF-specific cobalamin binding was found in the parietal cells of man, monkey, rabbit, guinea pig, cat and ox (Hoedemaeker et al. 1964, 1966). Moreover, these studies demonstrated IF in the peptic cells of rat and mouse, and in the pyloric and duodenal glands of the hog. Localisation using IF antibodies has confirmed these production sites for human IF (Glass, 1974; Smolka & Donaldson, 1990; Levine et al. 1980). Monoclonal antibodies against human IF colocalised IF and H,K-ATPase in parietal cells in the hog fundus, as well as in human mucosa (Smolka & Donaldson, 1990). This location in hog gastric mucosa agreed with findings using polyclonal antibodies raised against rat IF (Lee et al. 1989). These studies confirmed older studies (Glass, 1974) showing that IF antibodies were not species specific. IF expression by nonparietal cells has been reported in some species. In the dog IF is found mostly in pancreatic duct cells, but it is also located in enteroendocrine cells and salivary duct cells as well as in parietal cells (Vaillant et al. 1990). In the rat the major source is gastric chief cells (Schepp et al. 1983*a*; Dieckgraefe et al. 1988), although by immunohistochemistry (Lee et al. 1989) and in situ hybridisation (Dieckgraefe et al. 1988; Maeda et al. 1995) parietal cells also express small

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amounts of the protein. In samples of isolated gastric mucosal cells the fractions with estimated 0% parietal cells appear to contain about 15% of maximal IF binding activity (Schepp et al. 1983*a*), although clearly the degree of cobalamin binding activity is correlated with the percentage of chief cells.

In human gastric mucosa a systematic examination of multiple sites was not achieved in previous studies due to the lack of tissue availability. Biopsy material was used from only the gastric body in most studies (Levine et al. 1980, Smolka & Donaldson, 1990), and in one study the location in the fundus and antrum was approximated by the use of the Quinton tube (Lee et al. 1989). In this latter study only parietal cells were identified as a source of IF by immunomicroscopy at the light and ultrastructural levels, using peroxidase-linked detection systems.

Because of the diversity of IF expressing cells in some nonhuman species, we reexamined the cells expressing IF in 6 different locations within the human gastric mucosa, using an antibody raised against recombinant human IF, and including the more sensitive immunogold electron microscopy. Our observations confirm the parietal cell as the major source of IF in normal human gastric mucosa, but indicate also a complex and redundant expression of IF in small clusters of cells at the margins of the anatomical subdivisions of the stomach, mainly gastric chief and enteroendocrine cells. These findings show that the potential for IF expression in human stomach is not limited to the parietal cell.

MATERIALS AND METHODS

Tissue

Four whole stomachs were obtained from transplant donors (2 male, 2 female, age range 19–47 y). The stomachs were arterially perfused with Wisconsin solution and maintained in the same solution until opened, usually within a few hours. After opening along the greater curvature sections were removed from 6 areas, located by visual inspection: the cardia near the oesophageal junction, the junction of cardia and fundus, the junction of fundus and body, the midbody, the junction of body and antrum, and the antrum. Three full thickness sections, each 2 × 2 cm in mucosal area, were obtained from all areas of each stomach. Tissue biopsies were taken from the same areas in patients undergoing diagnostic upper endoscopy and whose mucosa was normal in appearance and by subsequent histological examination. Permission to obtain both types of tissue was approved by the Human Studies Committee of Washington University School of Medicine. For the study using rat stomach the glandular portion of the stomach was removed from a fasting animal anaesthetised with methoxyflurane.

Fixation

Samples for light microscopic immunoperoxidase cytochemistry from all transplant donor stomachs were fixed in Zamboni's fixative (picric acid-paraformaldehyde). Those samples from endoscopic biopsy were first fixed in 10% neutral formalin, then transferred to Zamboni's fixative and embedded in paraffin. Donor gastric tissues for immunoelectron microscopy were processed according to a modification of the procedure of Berryman & Rodewald (1990). They were fixed for 2–3 h in 4% paraformaldehyde, 0.2% tannic acid, 0.5 mM $CaCl_2$ in 100 mM phosphate buffer, pH 7.4. The samples were then rinsed several times at 0 °C (over 2 h) with 3.5% sucrose in 100 mM phosphate buffer containing 0.5 mM NH_4Cl in the sucrose/phosphate buffer for 1 h on ice. Maleate buffer, 100 mM, containing 3.5% sucrose, pH 6.5, was used as a rinse to remove phosphate ions. Five buffer changes were made over 1 h. Staining in block with 2% uranyl acetate in the maleate/sucrose buffer (pH 6.0) was carried out for 2 h on ice in the dark. Tissues were dehydrated through a graded series of cold acetone and embedded in LR Gold. Blocks were polymerised by u.v. light at −20 °C.

Antibodies and Western blots

Rabbit antihuman polyclonal antiserum was raised against recombinant IF produced in baculovirus-infected Sf9 cells (Gordon et al. 1992). The antiserum reacted with a 50 kDa protein in Western blots of human gastric mucosal homogenates, which was slightly larger than recombinant human IF produced in baculovirus (Fig. 1). In addition, a much less abundant second band of about 60 kDa was present. This larger protein may be the result of different glycosylation of IF, as such differences are found in recombinant compared with native human IF (Gordon et al. 1995). Rabbit antihuman human pepsinogen was obtained from Dr Michael Samloff (Dept. of Medicine, UCLA Medical School). Rabbit antihuman gastrin was purchased from Dako Corporation (Carpinteria, CA).

Immunocytochemistry

The Vector-Elite kit (Vector Labs, Burlingame, CA) was used to stain the paraffin sections. Optimal

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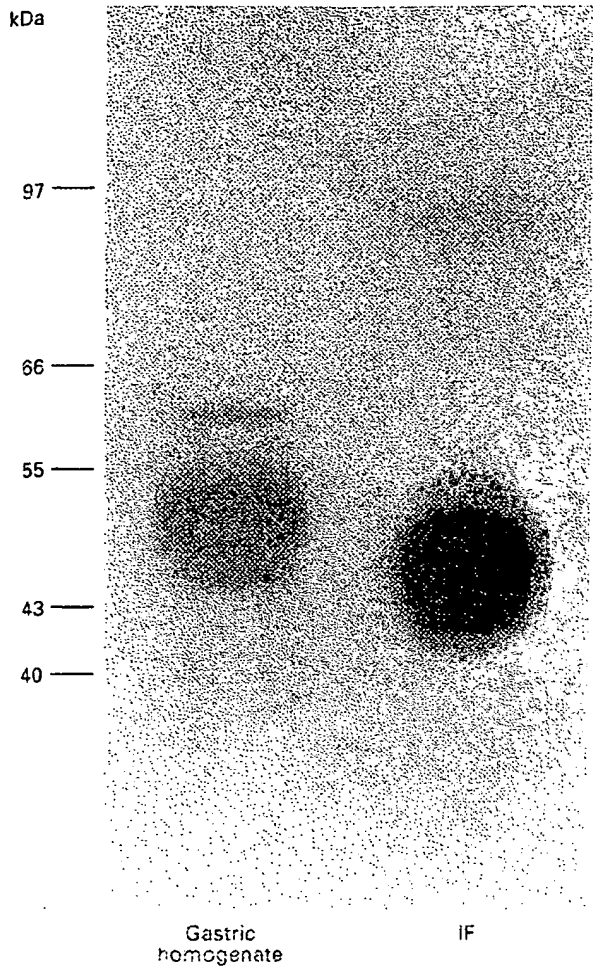

Fig. 1. Western blot of human gastric mucosal homogenate using antiserum raised against recombinant intrinsic factor (IF). First antibody was used at a 1:2000 dilution, with 2nd antibody at 1:5000. 10 μg of gastric mucosal homogenate were applied to the acrylamide gel. The position of recombinant IF (5 ng) was used as a marker. The gel was developed using the enhanced chemiluminescence (ECL) detection system (Amersham, Downer's Grove, IL) as described previously (Gordon et al. 1992).

staining was obtained with the primary antiserum at a 1:200 dilution. Sections photographed were not counterstained to allow easy identification of the brown stain on black and white reproductions. However, adjacent sections were counterstained with haematoxylin and eosin to allow confirmation of the stained cell type. In some experiments double labelled immunofluorescence was used to identify cells positive for IF and either pepsinogen or gastrin, as markers of chief cells or enteroendocrine cells. The second antibodies used were goat antirabbit IgG-indocarbocyanine (Cy3) (Jackson ImmunoResearch, West Grove, PA) and goat antirabbit IgG-fluorescein isothiocyanate (FITC) (Sigma Chemical Corp, St Louis, MO). Black and white reproductions were made from the green labelled IF positive coloured slides and the red positive slides (pepsinogen or gastrin) and placed side by side, so that IF production could be clearly identified in nonparietal cells.

Immunogold cytochemistry on transmission electron microscopy

Silver/gold sections of the samples were incubated with the antiserum, washed with 100 mM Tris buffer containing 250 mM NaCl, 5 mg/ml bovine serum albumin, and 0.05% Nonidet P-40, and then incubated with protein A-gold, 15 nm (E-Y Laboratories, Inc., San Mateo, CA). The reactions were carried out for 2 h at 22 °C or overnight at 4 °C, with the antibodies and the protein A-gold at various dilutions. The sections were rinsed, then vapour-osmicated for 30 min and stained with uranyl acetate and Reynold's lead citrate. The anti-IF serum was either omitted or preabsorbed with IF at equivalence (as determined by Ouchterlony double diffusion) in the control samples. Samples were viewed and photographed in a Philips CM-12 transmission electron microscope at 60 kV.

RESULTS

Immunohistochemistry

Three sections from each of the 6 regions sampled were examined in all 4 donor stomachs. Each stomach showed areas of IF production in nonparietal cells, as well as in the majority of the parietal cells. The Table documents in each stomach the cell type involved in the sections containing such cells. Mucous cells in the gastric cardia (Fig. 2*a–c*) and in other gastric regions (data not shown) did not show positive staining for IF

Table. *Distribution of intrinsic factor (IF) positive nonparietal cells in human stomachs*

Area	Cell stained	Nonparietal stomach*			
		No. of sections positive for IF in nonparietal cells			
		1	2	3	4
Cardia	C, EE	1/3	2/3	1/3	3/3
Cardiac/fundal junction	C	3/3	3/3	2/3	3/3
Fundal/body junction	C	2/3	2/3	2/3	3/3
Body	—	0/3	0/3	0/3	0/3
Body/antral junction	EE	2/3	3/3	2/3	3/3
Antrum	—	0/3	0/3	0/3	0/3

* Four intact stomachs were obtained from transplant donors. Three samples (1 × 1 cm) were excised from each area and duplicate sections were stained from each sample. The results correspond with the number of sections positive for IF from each section of the 4 stomachs. C, chief cell; EE, enteroendocrine cell.

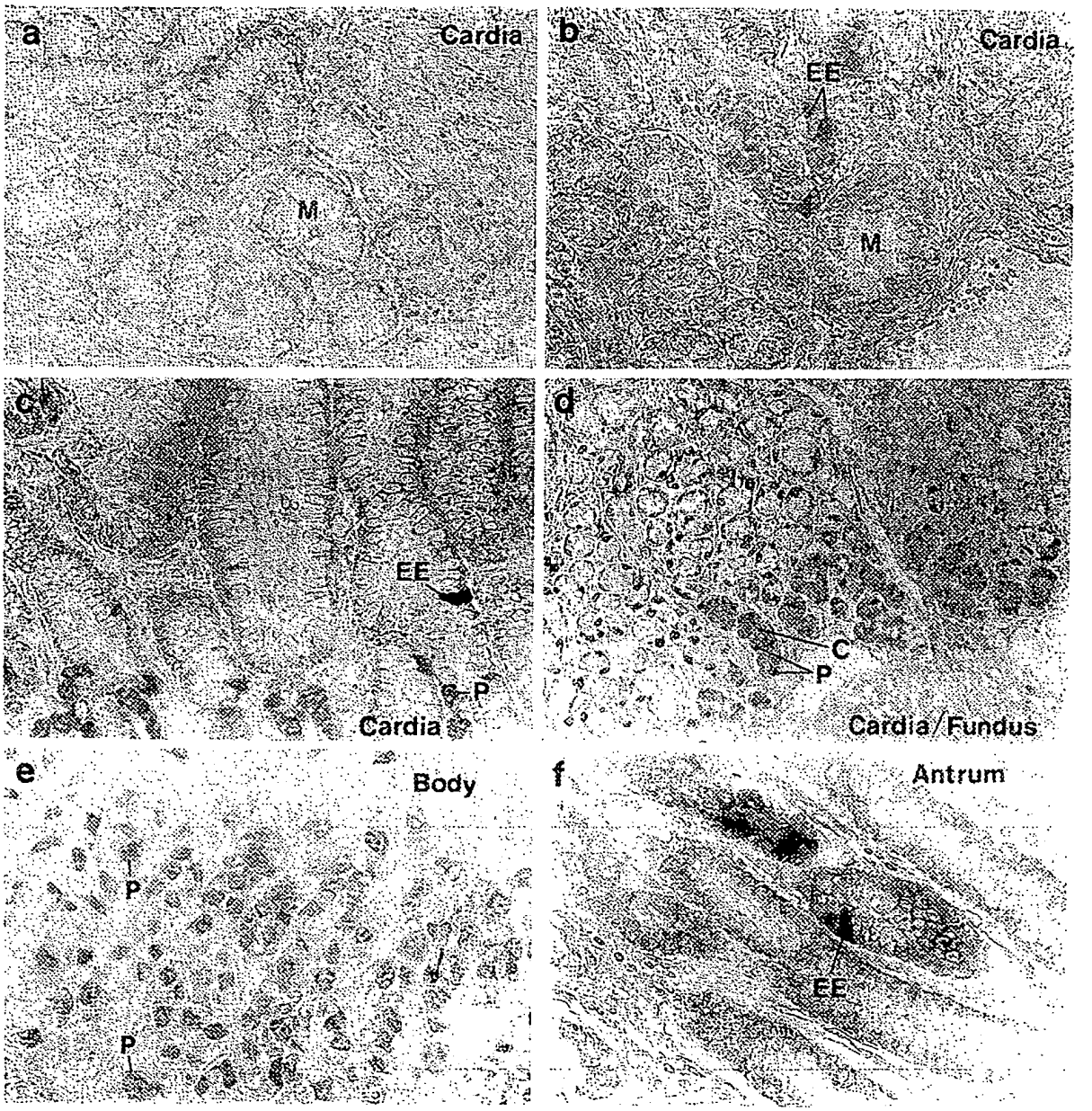

Fig. 2. Intrinsic factor localisation in human gastric mucosa from transplant donors. IF antiserum was used in a 1:200 dilution. In each instance duplicate slides cut from the same block demonstrated identical changes. (*a*) Cardia from subject 1 showing absence of mucous cell staining (× 200); (*b*) cardia from subject 2 showing occasional enteroendocrine cells positive for IF (× 400); (*c*) cardia from subject 3 showing occasional positive enteroendocrine cells (× 400); (*d*) cardiac/fundal junction from subject 2 showing clusters of chief cells positive for IF (× 100); (*e*) body from subject 3 showing parietal cell positivity (× 200); (*f*) antrum from subject 4 showing occasional enteroendocrine cell staining (× 400). P, parietal cell; C, chief cell; EE, enteroendocrine cell; M, mucous gland.

when detected with the Vector-Elite kit. Parietal cell staining was generalised in the sections of the gastric cardia and fundus that were deep to the mucous cells (Fig. 2*c*, *d*), and also in the gastric body (Fig. 2*e*). Subpopulations of chief cells showed positive staining for IF, especially distributed along the lower two-thirds of the epithelium (the normal location for chief cells) (Fig. 2*d*). All staining over chief cells was eliminated by addition of 1μg of purified IF to the incubation with first antibody (see Fig. 6 for the lack of reactivity using serum preadsorbed with IF).

Positively stained cells observed in both the cardia (Fig. 2*b*, *c*) and antrum (Fig. 2*f*) matched the shape and distribution of enteroendocrine cells. No identifiable parietal cells were seen in most sections of the gastric antrum (Fig. 2*f*). Identification of chief (zymogenic) and enteroendocrine cells containing IF was confirmed by double labelled immunofluorescence studies. Using antiserum against gastrin, cells were found in the antrum that stained positively with Cy3 (red) and appeared to correspond with enteroendocrine cells (Fig. 3*A*). These cells were also

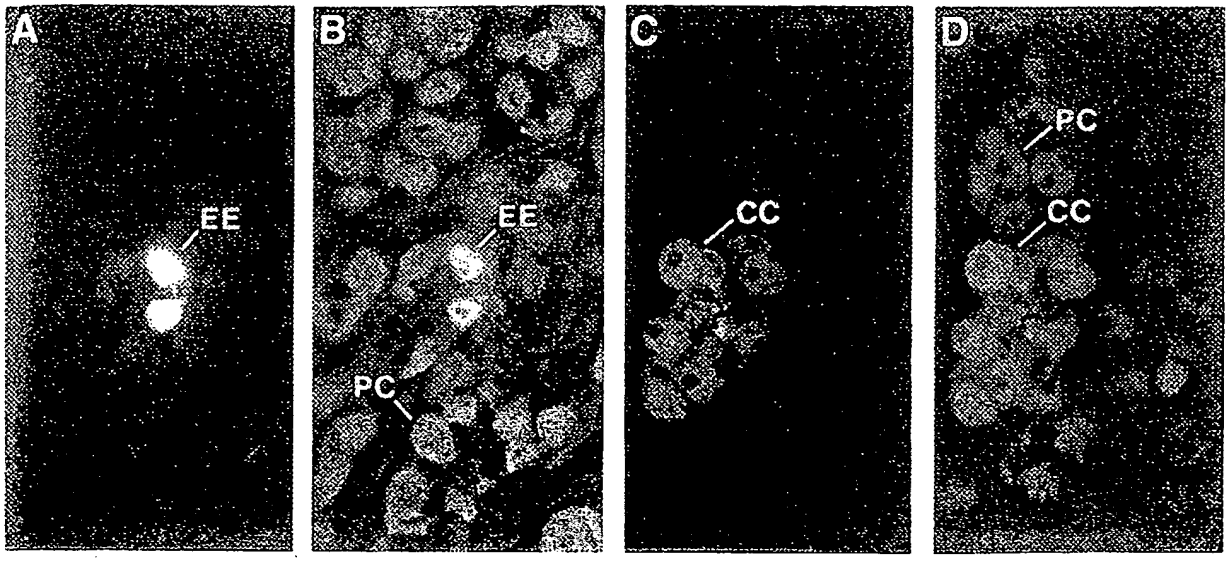

Fig. 3. Fluorescent immunochemistry of human gastric mucosa. Samples from subject 2 were used. Panel *A* shows staining using antiserum against gastrin, panels *B* and *D* using antiserum against intrinsic factor, and panel *C* using antiserum against pepsinogen. Note the presence of both IF and pepsinogen in the chief cells (CC) identified in the fundal/body junction in panel *C*, and of both IF and gastrin in the enteroendocrine cells (EC) identified at the body/antral junction in panel *A* (×200).

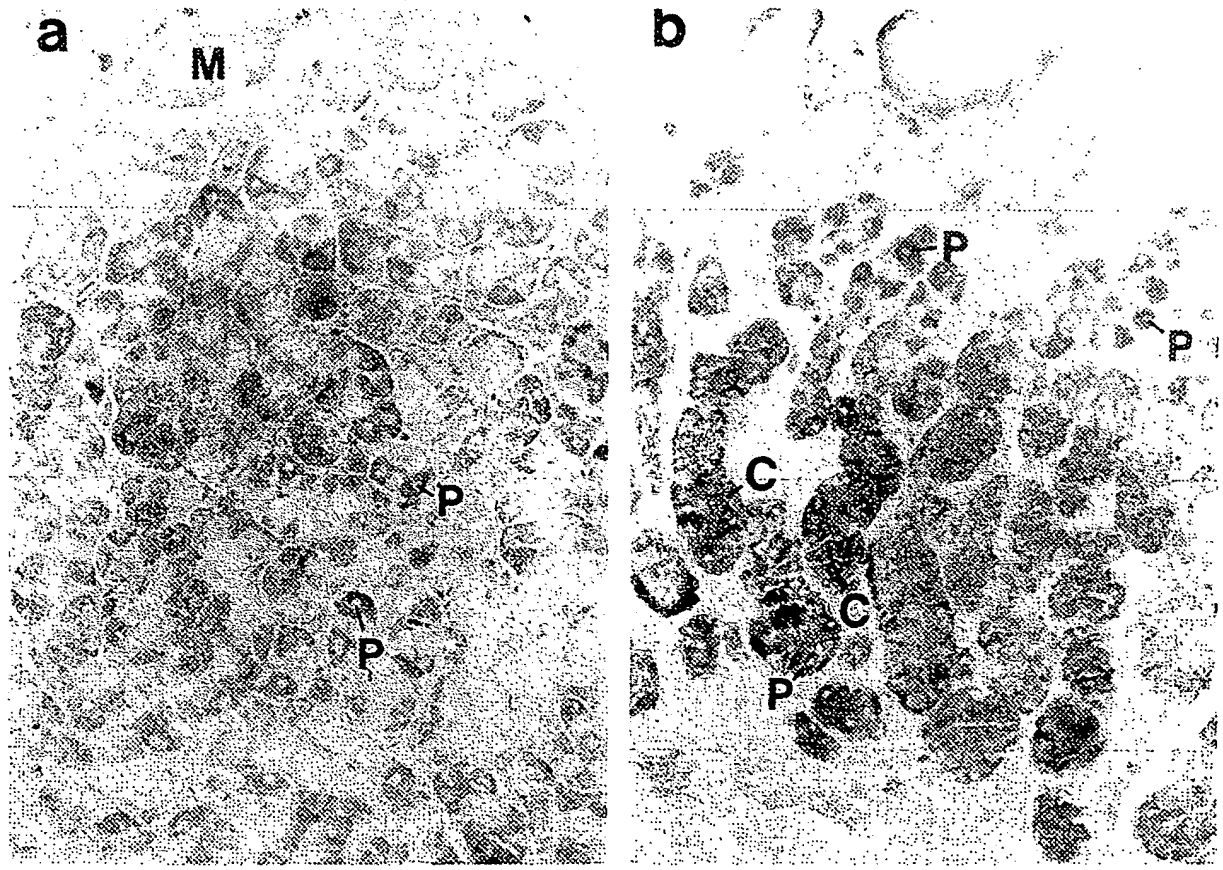

Fig. 4. Intrinsic factor localisation in human gastric mucosa obtained from endoscopic biopsy. Sections were treated and labelled as described in Figure 2. (*a*) Gastric body; (*b*) cardiac/fundal junction from same subject. Note the clusters of positively stained cells in panel *b* (×200).

stained positively with FITC (green) using antiserum against intrinsic factor (Fig. 3*B*), and the cells stained yellow when observed at both wavelengths, supporting the presence of intrinsic factor in these enteroendocrine cells. In the gastric body cells at the base of the gland stained positively with Cy3 (red) using antiserum against pepsinogen (Fig. 3*C*). These cells and other cells stained green (FITC) using

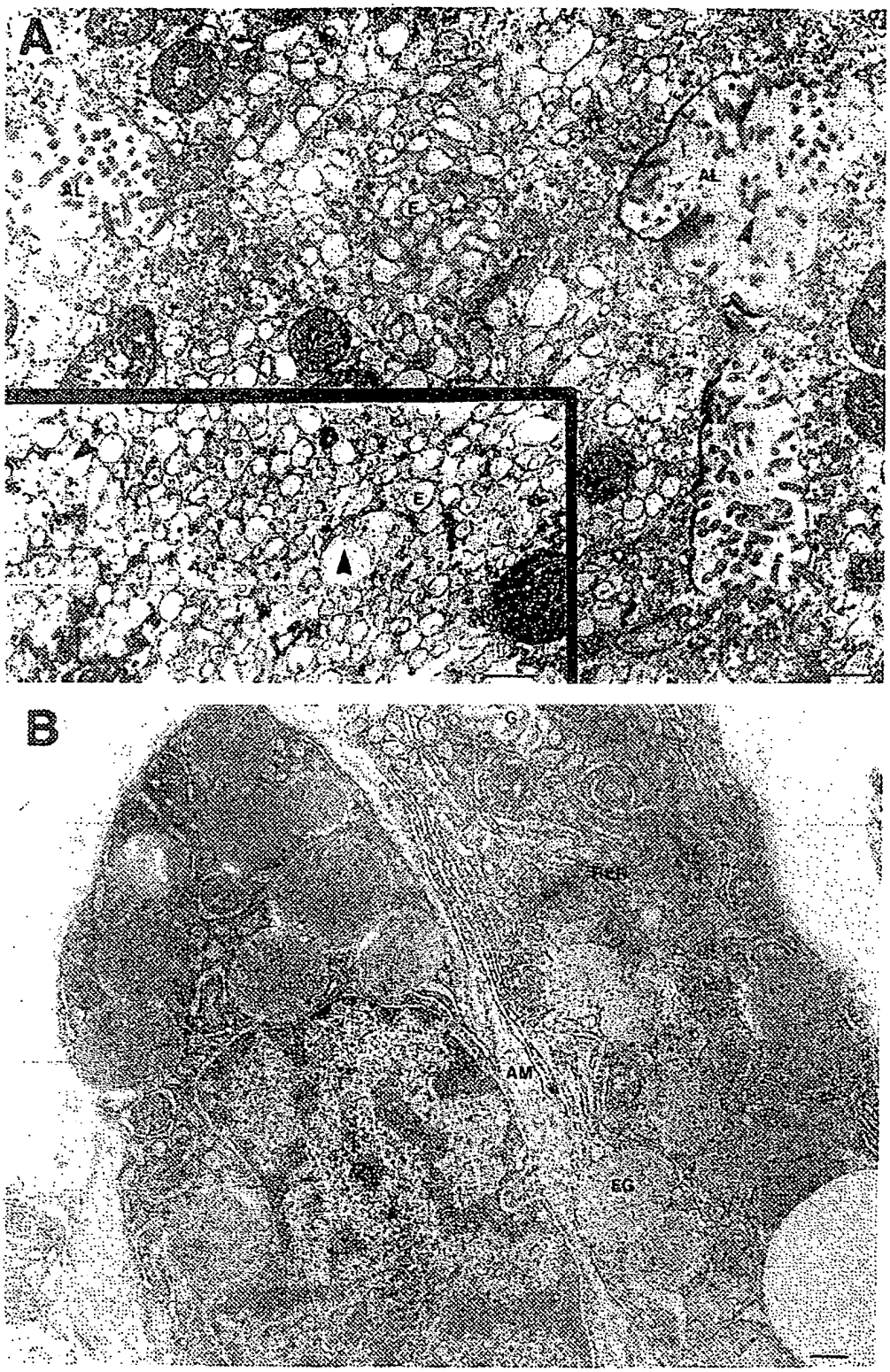

Fig. 5*A*, *B*. For legend see opposite.

antiserum against intrinsic factor (Fig. 3*D*). The commonly stained cells appeared yellow when observed at both wavelengths, and appeared to represent chief cells that expressed intrinsic factor.

Similar patterns were seen in samples obtained from endoscopic biopsies. When samples from the gastric body were examined from patients with normal histological biopsies, only parietal cells stained positively for IF (Fig. 4*a*). In 2 of 4 patients, samples from the junction of the cardia and fundus demonstrated clusters of cells near the base of the gastric glands that occupied the position of chief cells (e.g. Fig. 4*b*).

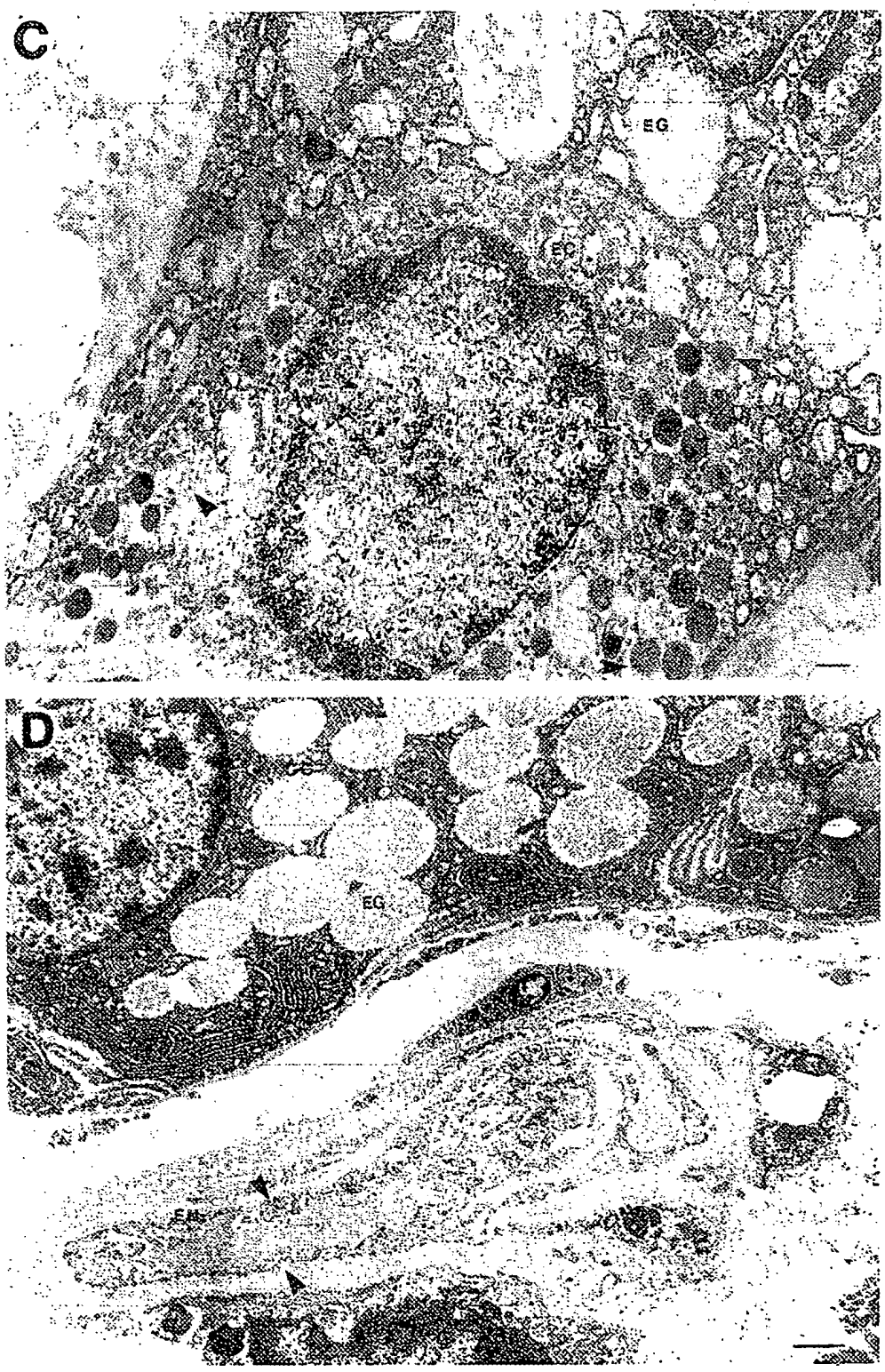

Fig. 5. Immunoelectron microscopy of human gastric mucosa using IF antiserum. Sections illustrated were prepared from a single stomach and stained with antiserum against IF as described in Methods. (*A*) Parietal cells. Two apical lumina are shown (AL); an accumulation of endosomes (E) is shown in the inset. Notice the luminal labelling, both in the lumen and over the projecting microvilli. The endosomal membranes and lumina are also IF-positive (arrowheads). Mitochondria (M) and cytoplasm are both negative. (*B*) Chief cells. Two adjacent cells with different labelling densities are shown. The exocrine granules (EG) as well as the apical membranes (AM) label for IF. The Golgi (G) is IF-positive, but there is very little labelling of the rough endoplasmic reticulum (RER). (*C*) Enteroendocrine cell (EC). Secretory granules (SG) and areas of cytoplasm are IF-positive (arrowheads). The adjacent chief cell has degranulated, and the empty exocrine granules are negative. (*D*) Endothelial cell (EN). The cell membrane of the endothelial cell is IF-positive (arrowheads). An adjacent chief cell (CC) exhibits a low level of exocrine granule (EG) labelling. Bar, 0.5 μm.

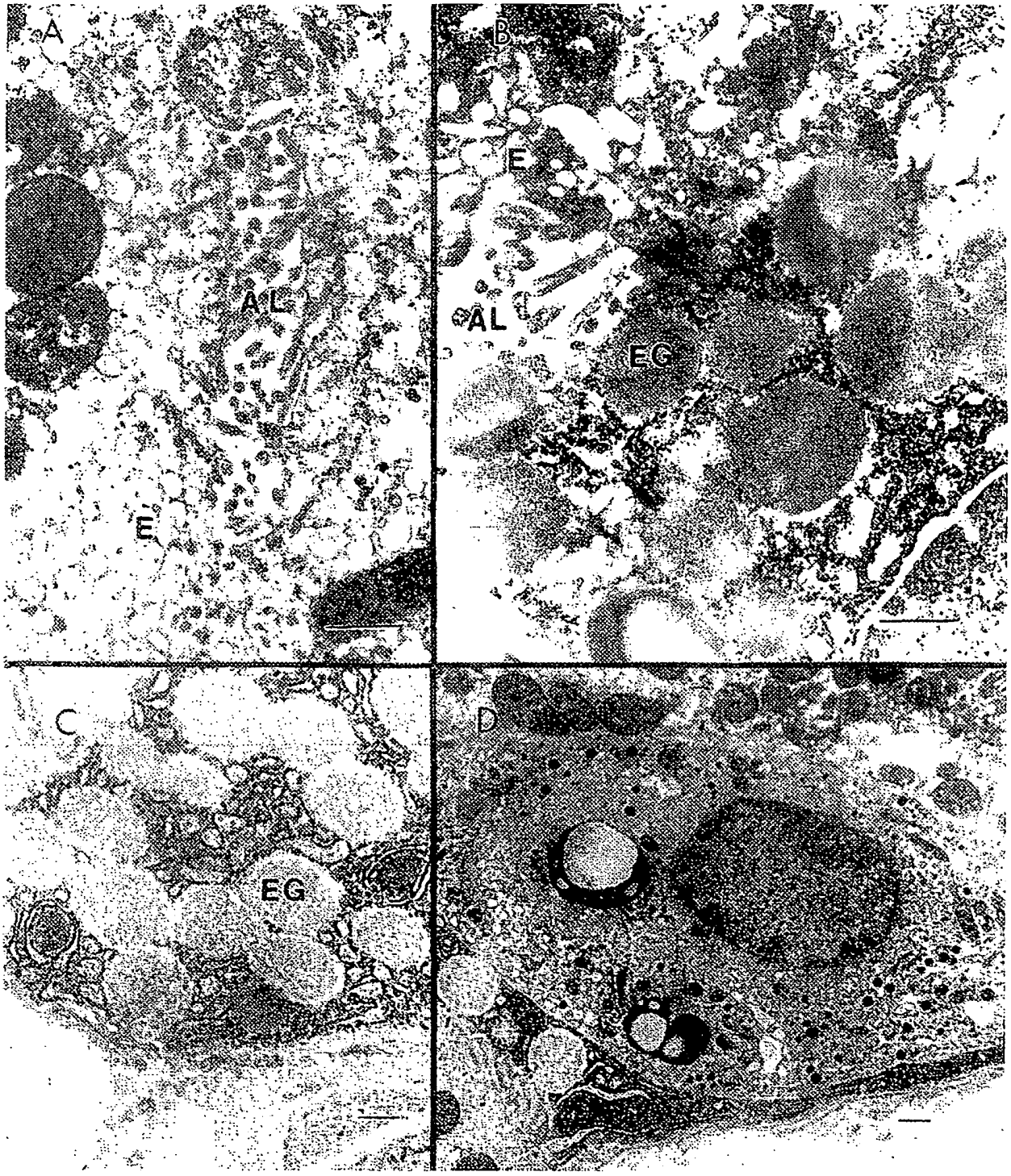

Fig. 6. Immunoelectron microscopy of human gastric mucosa using preadsorbed IF antiserum. The primary antiserum was preadsorbed with IF at equivalence and the processing performed as described in Methods. (*A*) Human parietal cell. (*B*) Rat parietal and chief cells, included as a negative control for adsorption of chief cell staining. (*C*) Human chief cell. (*D*) Human enteroendocrine cell. AL, apical lumen; E, endosome; EG, exocrine granule. Bar, 0.5 μm.

Immunoelectron microscopy

Parietal cells. The most intense labelling was observed over parietal cells, all of which were positive for IF. The highest antigen densities were displayed by endosomes and apical lumina (Fig. 5*A*). Although most of the endosomal labelling was membrane-associated, there was some localisation of the antigen to the lumen. The labelling over apical lumina was typically associated with microvilli, but there was also

immunoreactivity in cellular debris and secreted material in the lumina.

Chief cells. Figure 5*B* demonstrates 1 cell with each of 2 different labelling densities, variable and intense. The antigen was localised to exocrine granules, apical membranes, and to a lesser extent Golgi stacks. The contents of exocrine granules from IF-positive cells in the process of degranulation retained their antigenicity.

Enteroendocrine cells. The secretory (exocrine) granules and cytoplasm of some enteroendocrine cells were IF-positive (Fig. 5*C*). Some staining of the nucleus was seen in occasional cells, including this one, but this staining was competitively inhibited with IF.

Endothelial cells. The plasma membranes and smooth surfaced vesicular compartments of some endothelial cells in the lamina propria labelled for IF (Fig. 5*D*). Labelling in these cells was fairly uniform, but such cells were not uniformly distributed within the stomach, all being found in areas near enteroendocrine cells (antrum and cardia).

Controls. For controls the anti-IF serum was either omitted or preabsorbed with IF at equivalence. Figure 6 shows data using preadsorbed antiserum. None of the structures in either human parietal cells (Fig. 6*A*), chief cells (Fig. 6*C*), or enteroendocrine cells (Fig. 6*D*) was decorated with gold particles. A parietal and chief cell from rat gastric mucosa are included (Fig. 6*B*) to demonstrate the effectiveness of preadsorbed antiserum in a different species in which the chief cell is the principal site of IF production.

DISCUSSION

The present study identified in the human stomach the presence of IF in most gastric cell types previously reported in various species (parietal, chief, enteroendocrine), and also in endothelial cells. Early studies on the distribution of IF in mammalian stomach used radiolabelled cobalamin to bind cellular IF, and the complexes were detected autoradiographically (Hoedemaeker et al. 1964, 1966). Autoradiographic localisation, however, presents the disadvantage of errors in point resolution, because of the length of the radioactive decay paths. More recently, specific antibodies against IF have been used, but the detection system employed was peroxidase based (Lee et al. 1989). This system tends to obscure cellular features and the reaction product can diffuse on tissue sections. The immunogold detection system was chosen for EM localisation in the present study because it provides quantifiable and point localisation of the antigen. Finally, the present study systematically examined 6 different regions in the human stomach, and could obtain large amounts of material, whereas previous studies had used few sites, largely from the gastric body (Glass, 1974; Levine et al. 1980; Smolka & Donaldson, 1990).

The parietal cell localisation observed in the present study corresponds to the previously reported patterns for human parietal cells, in that most cells were in the gastric body, located in the midzone of the crypt (Hoshiko, 1953), even in fetal and neonatal human stomach (Aitchison & Brown, 1988). The ultrastructural localisation of IF within the parietal cell also corresponds with that previously reported (Levine et al. 1980, 1981). IF has been localised to chief cells in rats (Hoedemaeker et al. 1964, 1966; Schepp et al. 1983*a*, *b*), mice (Hoedemaeker et al. 1964, 1966), and dogs (Vaillant et al. 1990; Simpson et al. 1993), but has not been reported in human chief cells before. The chief cell staining at the light level was not so intense as the parietal cell staining, but was quite definite. The labelling was reversed by addition of IF at both the light and transmission electron microscopic level, and appears to be specific. Moreover, the gold particle labelling of exocrine granules within the cell also suggests specificity, as IF is secreted from chief cells, at least in the rat (Schepp et al. 1983*a*), and localisation to secretory granules would be expected. The variability in IF content of chief cells and the infrequency of its occurrence may be due to many factors, including loss of antigen during fixation, lower levels of IF synthesis in fasting gastric mucosa, true mosaicism as reported in mammalian small intestine (Rubin et al. 1989; Mauiri et al. 1991), or to characteristics of gene expression unique to the gastric mucosa (see below). Previous studies in animals have shown that the presence of IF in mucosal cells detected by immunohistochemistry was reflected in the mRNA content of such cells examined by in situ hybridisation, whether the cells were in the stomach (Dieckgraefe et al. 1988; Maeda et al. 1995) or in the pancreas (Simpson et al. 1993). Such studies are needed to confirm this conclusion in human stomach.

Positive gold labelling over secretory granules on immunoelectron microscopy and double labelling with antigastrin or antipepsinogen antiserum supports specificity of localisation in nonparietal cells. An intriguing finding was the occasional positive endothelial cell, often underlying chief or enteroendocrine cells. Secretory products of gastric chief cells (e.g. pepsinogen) or of gastrointestinal enteroendocrine cells (gastrin in particular from G-cells) are

released basolaterally and enter the blood (Kobayashi & Sasagawa, 1976; Walsh, 1994). The positive labelling for IF could represent IF in transit to the circulation from enteroendocrine cells. The lack of positive endothelial cells in other areas of the lamina propria (not beneath chief or enteroendocrine cells) supports this suggestion. Basolateral secretion of IF from enteroendocrine cells in the stomach could explain the small amount of IF that appears in the urine (Wahlstedt & Grasbeck, 1985). The source of this IF had been considered to be IF that was absorbed from the lumen, but intact IF does not cross a cell monolayer in vitro (Ramanujam et al. 1991; Gordon et al. 1995).

An intriguing observation was the clustering of ectopic IF production in cells at the edges of anatomical sections of the stomach, i.e. oesophageal/cardiac junction, cardiac/fundal junction, body/antral junction. This finding recalls the distribution of 'ectopic' parietal cells in the human antral mucosa, occurring most often in the region just proximal to the gastroduodenal junction (Tominaga, 1975). Parietal cells are found also in the antrum of fetal and newborn stomachs, but largely disappear in most specimens during the 3rd trimester of pregnancy (Aitchison & Brown, 1988; Kelly et al. 1993). Thus the finding is probably not related to metaplasia, but may indicate that in transitional zones of gastric mucosa, definition of cell lineage and gene expression within those cells may be subject to multiple factors.

The epithelium of the mammalian stomach contains 4 major epithelial cell lineages: pit/surface mucous cells, parietal (oxyntic) cells, chief (zymogenic) cells, and enteroendocrine cells. In addition to the surface mucous cells, the major regions in the human stomach contain a principal cell type(s): mucous glands in the cardia, parietal and chief cells in the fundus/body, and mucous glands and enteroendocrine cells in the antrum. Much of the data on cell lineage formation comes from the mouse stomach and the studies of Karam & Leblond (1993*a–d*). Their studies demonstrated that parietal cells are supplied from multiple cell lineages, but chief cells derive mostly from preneck cell precursors. 24% of undifferentiated granule-free cells give rise to preneck cell precursors, but about 2% of these precursor cells can give rise to parietal cells (Karam & Leblond, 1993*c*). Thus an individual cell's commitment to its final lineage can be affected at various sequential steps within the gastric glands, and some apparently similar precursor cells may evolve into either chief or parietal cells.

Although IF is apparently confined to zymogenic (chief) cells in the mouse, the 5′-nontranscribed domain of the mouse IF gene from −1029 to +55 directs expression of the human growth hormone transgene only to parietal cells (Lorenz & Gordon, 1993). This finding suggests that there may be cis-acting elements missing in this transgene that normally act to suppress IF expression in parietal cells in the mouse. Viewed another way, the presumed presence of such silencer regions could account for the occasional expression of IF in cells at the margins of anatomical regions within the stomach where the microenvironment for expression (nuclear proteins, cytokines, etc.) might impair the full activity of such silencer regions. This possibility can be tested experimentally.

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